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Short Review

Light-harvesting and structural organization of Photosystem II: From individual complexes to thylakoid membrane

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ABSTRACT

Photosystem II (PSII) is responsible for the water oxidation in photosynthesis and it consists of many proteins and pigment-protein complexes in a variable composition, depending on environmental conditions. Sunlight-induced charge separation lies at the basis of the photochemical reactions and it occurs in the reaction center (RC). The RC is located in the PSII core which also contains light-harvesting complexes CP43 and CP47. The PSII core of plants is surrounded by external light-harvesting complexes (lhcs) forming supercomplexes, which together with additional external lhcs, are located in the thylakoid membrane where they perform their functions.

In this paper we provide an overview of the available information on the structure and organization of pigment-protein complexes in PSII and relate this to experimental and theoretical results on excitation energy transfer (EET) and charge separation (CS). This is done for different subcomplexes, supercomplexes, PSII membranes and thylakoid membranes. Differences in experimental and theoretical results are discussed and the question is addressed how results and models for individual complexes relate to the results on larger systems. It is shown that it is still very difficult to combine all available results into one comprehensive picture.

Contents

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1. Introduction

The light-driven reactions that take place in Photosystem II (PSII) of green plants, algae and cyanobacteria lead to the oxidation of water, the reduction of plastoquinone and the formation of a proton gradient across the thylakoid membrane. PSII forms a tandem with PSI and together they drive $NADP^+$ reduction with H_2O as electron donor [1].

Fig. 1A shows a model of a PSII supercomplex of plants. PSII supercomplex refers to complexes composed of a PSII core, where the photochemistry takes place, and the outer light-harvesting complex (Lhc) system which contains most of the sunlight-absorbing pigments of PSII and that provides the core with excitation energy. PSII core complexes contain around 20 different subunits [2,3] that have only slightly changed during evolution going from cyanobacteria to higher plants. Light absorption in the outer antenna is followed by excitation energy transfer (EET) to the pigment-protein complexes CP43 and CP47 in the core, which in turn transfer excitations to pigments in the reaction center (RC). The excitation of the primary donor P680 leads to electron transfer to a nearby pheophytin (Pheo) [4–7], that is followed by electron transfer via plastoquinone Q_A to plastoquinone Q_B, although also recombination of charges can take place [8]. It was also more recently demonstrated that two different pathways for charge separation exist [9,10]) as was suggested earlier by Van Brederode and coworkers [11,12]. The thus created primary cation radical $P680^{+\cdot}$ has an E_m value of +1.25 V [13] which is far higher than the value of +0.80 for Chl in solution [14]. Reduction of $P680^{+\cdot}$ proceeds via a redox-active tyrosine of the D1 protein (D1 and D2 proteins constitute the RC) and a cluster of four manganese ions, which after the accumulation of four oxidizing equivalents oxidizes water to molecular oxygen [15,16].

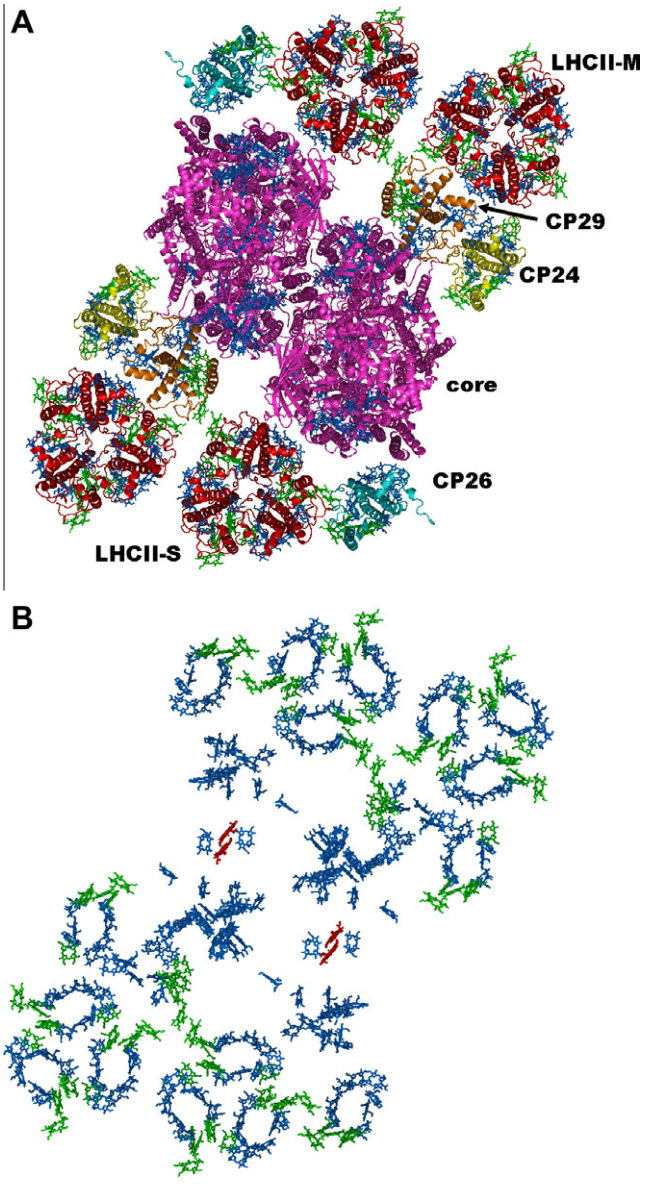

Fig. 1. *Model of the PSII supercomplex C2S2M2 from higher plants.* The model has been assembled based on [126] using the crystal structures of the cyanobacterial PSII core [44] (3BZ1 and 3BZ2) and LHCII trimer [78] (1RWT). (A) Model showing the protein organization. For the monomeric antennas, the structure of a monomeric LHCII has been used. Proteins of the core, magenta; LHCII, red; CP24, yellow; CP29, orange; CP26, cyan. (B) model shoring only the Chls organization. Chls a, green; Chls b, blue. The P Chls are in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The core is a rather expensive piece of machinery with its 20 different subunits whereas the amount of light-absorbing pigments is relatively low. In order to increase the absorption cross-section in a cost-effective way, plants and green algae have developed membrane-embedded light-harvesting complexes that form the outer antenna with a high pigment-to-protein ratio (35% of the mass is pigments), whereas cyanobacteria have the membrane-associated phycobilisomes (see e.g. [17]).

In higher plants six genes (*Lhcb1-6*) encode for the PSII antenna complexes [18]. Lhcb1-3 compose the light-harvesting complex II (LHCII) [19], the major antenna complex which is present in the membrane in the form of a trimer, while *Lhcb4-6* encode for the so called minor antennas, CP29, CP26 and CP24, respectively, that are present as monomers in the membrane [20,21]. These complexes contain the pigments Chl *a* and *b* and the xanthophylls lutein, violaxanthin and neoxanthin (except CP24) that absorb the sunlight. (Singlet) excitations are transferred from the carotenoids to the Chls and from Chls *b* to Chls *a*, and via a network of connected Chl *a* molecules the excitations finally arrive in the RC. Once in a while a Chl *a* singlet excitation is transformed into a Chl *a* triplet that could easily lead to the formation of destructive singlet oxygen molecules. Fortunately, these dangerous Chl triplets are nearly all (up to 95%) scavenged by the carotenoid molecules that are in Van der Waals contact with the Chl *a* molecules [22–26].

Whereas the outer antenna clearly increases the effectiveness of a PSII complex in dealing with the diluted photon flux in low-light conditions, in high-light conditions, there can easily be too many excitations to be handled by the photosynthetic machinery: The electron chain becomes blocked, leading to charge recombination which is often accompanied by spin inversion [27] and thus to dangerous triplet formation on the primary electron donor. Because the primary donor is not in direct contact with a protective carotenoid, this triplet can easily lead to the formation of singlet oxygen. An important way in which plants protect themselves against this threat is via the process of non photochemical quenching (NPQ): a mechanism that leads to a shortening of the PSII excited-state lifetime by introducing non photochemical quenchers in the outer antenna systems [28,29] thereby lowering the probability of singlet oxygen formation. This also implies that the Photosystem II supercomplexes need to be modular and flexible to be able to acclimate to different conditions.

In this review we will focus on the study of excitation energy transfer and charge separation (CS) in (parts of) PSII. Studies have been performed on isolated RC's, core preparations and light-harvesting complexes from different organisms but also on supercomplexes and different types of membranes. It will be discussed to which extent the different results (dis)agree with each other. For instance, there may be differences between complexes from differ-

ent organisms but it can also happen that specific complexes have different properties when they occur in isolated form or in larger functional complexes. Moreover, the properties can change in different environmental conditions and in addition reorganizations in the membrane may occur. It will become clear that there is still a lot of both implicit and explicit disagreement between different research groups about the various experimental results and their interpretation. Solving the discrepancies is not only important for a proper understanding of the EET- and CS-mechanism(s) in PSII but also for determining the efficiency of the trapping process of excitations and for understanding the process of NPQ. Moreover, a proper understanding of the various kinetic parameters and events will be instrumental for the interpretation of ps measurements *in vivo* [30–32]) under various stress conditions. We will end this review with some open questions and suggestions for future research. We would also like to refer to various other reviews that have appeared in recent years discussing more/other aspects of PSII [2,3,33–38].

2. Some basic concepts

For the topics that will be discussed in this review it is helpful to realize that the following relation holds for ϕ_{CS}, the quantum efficiency of charge separation in PSII:

$$\phi_{CS} = 1 - \tau/\tau_{Chl},$$

where τ and τ_{Chl} are the total (average) excited-state lifetime of an excitation in PSII in the presence and (the hypothetical) absence of charge separation, respectively. Whereas, the first lifetime can be measured directly, the second one is usually assumed to be equal to the average lifetime of an excitation in the antenna complexes. For Chl in isolated LHCII, the excited-state lifetime is ~4 ns [39] but it might be lower in the thylakoid membrane [40].

The total lifetime or overall trapping time τ can be considered as the sum of two contributions [41,42]:

$$\tau = \tau_{trap} + \tau_{mig},$$

in which the trapping time τ_{trap} represents the charge separation time from an excited-state equilibrated system. This is equal to the intrinsic charge separation time of the primary donor τ_{iCS}, divided by the probability that the excitation is actually located on the primary donor, and e.g. in the case of N isoenergetic pigments, including the primary donor, $\tau_{trap} = N\tau_{iCS}$ (when charge recombination is ignored). The migration time τ_{mig} corresponds to the time it takes for an excitation to reach the primary donor for the first time (also called first-passage time) and it reflects in fact the equilibration time. If τ is dominated by τ_{trap} then the charge-separation process is said to be trap-limited whereas it is called migration-limited in the reverse case.

The migration time is sometimes written as the sum of two terms, namely $\tau_{mig} + \tau_{del}$ [36] to take into account the fact that the excitation energy transfer step from nearby antenna pigments to the RC can be much slower than the transfer steps within the antenna. This is due to the fact that the RC pigments are relatively far away from the antenna pigments (see Fig. 2). This leads to the following equation:

$$\tau = \tau_{trap} + \tau_{mig} + \tau_{del},$$

where τ_{mig} is now the time needed to reach the pigments in the antenna that transfer energy to the RC and τ_{del} reflects the "delivery time" from these pigments to the RC, which also accounts for the probability that the excitation is located on the "delivery pigments". When τ_{del} is dominating the overall trapping time, then the charge-separation process is called transfer-to-the-trap limited. As will be discussed below, rather controversial opinions exist about the relative contributions of τ_{trap}, τ_{mig}, and τ_{del} to the overall lifetime, with important implications for the mechanism of EET but also for the mechanism of charge separation. Finally, the relative contributions of these terms can also be of relevance for the location of the quencher(s) participating in NPQ. If the charge-separation process in PSII is for instance trap-limited then it is irrelevant for the efficiency of NPQ where the quenchers are located. In that case excitation equilibration over PSII is extremely fast and decay of the excited state occurs mainly via CS with rate $k_{trap}(=1/\tau_{trap})$ and NPQ with rate k_{NPQ} (ignoring processes like fluorescence etc.) and the relative values of these rates determine the efficiency of either process. In case τ_{mig} (or τ_{del}) cannot be ignored then the efficiency of quenching will depend on the location of the quencher [43].

3. Structure and excited-state dynamics of the PSII core

3.1. The structure of PSII core

The structure of the PSII core from *Thermosynechococcus elongatus* at 2.9 Å resolution [44,45] shows the location of 35 Chls *a*, 2 pheophytins *a* (Pheo), 3 plastoquinones and 12 β-carotenes per

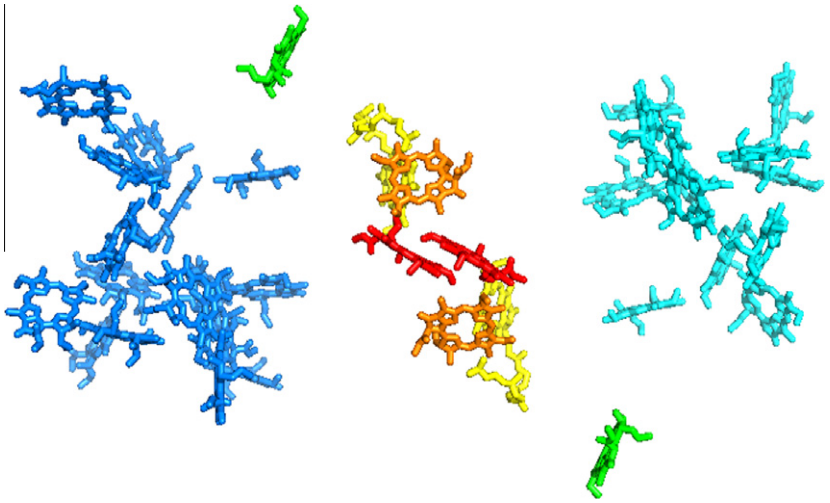

Fig. 2. *Chlorophyll organization in the core complex of PSII* [44]. Chls P, red; Chl D1 and D2, orange; Chlz green; Pheos, yellow. The Chls of CP47 are in blue and those of CP43 in cyan. The phytol chains of the Chls are omitted for clarity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

monomer in addition to the Mn cluster and other cofactors. The core is composed of four large integral membrane proteins, the products of genes PsbA–PsbD, which together contain 22 membrane-spanning helices and coordinate all the Chls present in the complex (Fig. 2). A number of small subunits account for 14 additional transmembrane helices (TMH). The products of genes *PsbA* and *PsbD* (complexes D1 and D2, respectively) form a heterodimer which coordinates 6 Chls, 2 Pheos and 2 plastoquinones (Q_A and Q_B), many of which participate in the electron transport chain. These complexes, together with the two proteins of $cytb_{559}$ form what is called the reaction center complex (RC). On the periplasmic side (lumenal side in higher plants) D1 provides most of the ligands for the Mn cluster which oxidizes water [46]. The cofactors of the electron transport chain are organized in two symmetric branches, with four Chls (P_{D1}, P_{D2}, Chl_{D1} and Chl_{D2}) located near the lumenal side of the membrane. It appears that in PSII all these four Chls are excitonically coupled with each other and with Pheo [47], whereas Chl_{D1} is the primary electron donor and $Pheo_{D1}$ the primary electron acceptor as shown by experimental measurements and theoretical calculations [6,7,9,10,48–50]. Two other Chls (Chlz D1 and Chlz D2) are probably involved in EET from CP43 and CP47 (see Fig. 2) [51,52]. *PsbB* and *psbC* encode for the two inner antenna complexes known as CP47 and CP43 respectively with the former being located on the side of D2 and the latter adjacent to D1. CP47 coordinates 16 Chls and CP43 13 Chls [45].

The structure of the PSII core from higher plants is not available at present but it is supposed to be very similar to that of cyanobacteria. The main difference in the protein composition is related to the extrinsic proteins involved in the stabilization of the water-splitting cluster [53] which do not coordinate pigments. The comparison of the primary structure of the conserved proteins of cyanobacteria and higher plants shows a very high degree of identity, thus suggesting that also the 3-D structures are very similar. This conclusion is supported by the analysis of the 5.5 Å projection map of the core from spinach (see [54] for full details). However, it is very important to keep in mind that the system is finely tuned and that even very small differences in the environment, especially around the cofactors of the electron transport chain, can influence the performance. A clear example of this is the presence of a glutamic acid in spinach and a glutamine in *T. elongatus* (*PsaA1 gene*) at position 130 of the D1 protein, substitution which has been shown to modulate the redox potential of pheophytin [55,56]. This indicates that, although the overall structure is conserved, extrapolation of the results obtained on the cyanobacterial system to higher plants requires some caution. This is particularly important considering that most of the studies on energy transfer and charge separation in the system were carried out on the PSII core of cyanobacteria and on the RC complex of higher plants, which can have different properties, especially concerning the free energy of the primary radical pair. It should be mentioned that the preparation of pure core complexes from plants that are still able to produce oxygen at a high rate is rather difficult because they tend to loose the subunits of the oxygen evolving complex far more easily than the core from cyanobacteria.

3.2. EET and charge separation in the PSII core

For many years the exciton/radical pair equilibrium model (ERPE model) [57–59] was used in photosynthesis research to analyze and explain a wide range of phenomena related to the kinetics of the early processes in PSII. It was partly based on time-resolved fluorescence and transient absorption measurements on PSII core particles. It is a trap-limited model that assumes that excitation equilibration in the core occurs on a time scale much faster than the overall trapping time. It was indeed concluded from studies on isolated CP43 and CP47 that excitation energy transfer within these complexes occurs on a time scale of only a few ps [60–62]. However, the distances between the pigments in these complexes and the ones in the RC (Fig. 2) are so large that it was suggested that the time for EET to the trap should give a substantial contribution to the overall trapping time [36,63–66]. More recently, Holzwarth and coworkers [7,67] after studying the core of *T. elongatus* with transient absorption and fluorescence measurements with increased time resolution, concluded from the presence of several decay processes faster than 10 ps and that precede the main decay process of 40 ps, that charge separation is nevertheless trap-limited. In a subsequent theoretical treatment of this issue by Raszewski and Renger [66] the core was split into three domains, namely CP43-$Chlz_{D1}$ (one of the peripheral RC pigments), CP47-$Chlz_{D2}$ and the RC. Generalized Förster theory was applied to model EET between different domains of strongly coupled pigments, and Redfield theory was used to describe intra-domain exciton relaxation. The authors concluded that the times of EET from CP43 and CP47 to the RC are 41 and 50 ps, respectively (back transfer times are 22 and 16 ps) and concomitantly that the experimental fluorescence decay can only be explained by ultrafast primary charge transfer (300 fs) from an excited-state equilibrated RC together with very slow charge recombination. Although the very fast energy transfer from CP43/CP47 to RC in the trap-limited model as found by Holzwarth and coworkers [7] can indeed not be explained by (generalized) Förster theory, it is on the other hand questionable whether the ultrafast primary charge separation in combination with very slow charge recombination (implying a very large initial drop in free energy) in the transfer-to-the-trap-limited model proposed by Raszewski and Renger [66] is realistic. At least in isolated RC complexes this has never been observed [6,7,48,69–74]. On the other hand, the results on PSII membranes could not be explained with the time-resolved results on isolated RC's [42]. The presence of a 50 ps transfer time from CP47 to RC as proposed by Raszewski and Renger is not supported by the conclusion of Andrizhiyevskaya et al. [75] that transfer from CP47 to RC in their RC-CP47 preparations from spinach occurs with a transfer time of at most 20 ps. The results of Raszewski and Renger [66] were also criticized by Jennings and coworkers [76]. These authors used a different approach, studying the excitation-wavelength dependence of the fluorescence decay of core particles from maize plants and they concluded that energy transfer to the RC could at most be 10–20% limiting. However, their average fluorescence lifetime (100–110 ps) was substantially longer than that of the other groups and it is also rather long when for instance compared to the lifetime of supercomplexes and PSII membranes (see below) that contain far more pigments but show only "slightly" longer lifetimes. In a different study [77] on core complexes from Chlorina, the lifetime as determined by the same research group was even longer: around 170 ps. The fact that the average lifetimes of core preparations from cyanobacteria are shorter may be due to the differences between the cores from plants and cyanobacteria but it may also be related to problems associated with the isolation of core preparations from plants (see above).

It is clear that there is a lot of controversy about the interpretation of the EET and charge-separation kinetics in the PSII core and as was already indicated, the disagreement extends to the comparison with results on isolated RC's and supercomplexes and PSII membranes.

4. Structure and excited-state dynamics of outer antenna complexes

4.1. Structure of the antenna complexes of Photosystem II

The antenna complexes of PSII of higher plants and green algae are composed of members of the lhc multigenic family. The

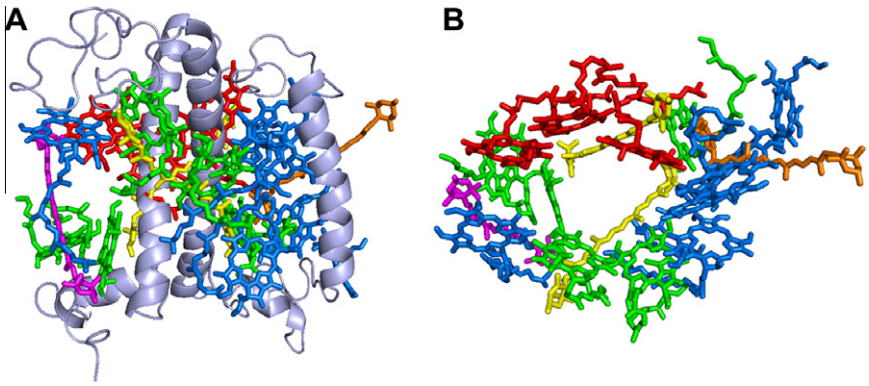

Fig. 3. *Model of the structure of LHCII* [78] (A) membrane view of the pigment-protein complex. (B) top view only showing the pigment organization. The protein is in light blue, Chls a in green, Chls b in blue, Chls 610-611-612 in red. Luteins, yellow; neoxanthin, orange; violaxanthin, magenta. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

structure of trimeric LHCII (Fig. 3A) has been obtained at 2.5–2.72 Å resolution [78,79]. Each monomer is composed of three transmembrane helices and two amphipathic helices and coordinates 14 Chl molecules (8 Chl *a* and 6 Chl *b*) and 4 xanthophylls (1 neoxanthin, 2 luteins and 1 violaxanthin) (Fig. 3B). Most of the Chls are coordinated by nucleophilic amino acids but a few others are coordinated via water molecules, the carboxylic group of the amino acids contributing to the backbone or even a lipid molecule. It has been shown that several of the binding sites can accommodate both Chl *a* or Chl *b* not only upon *in vitro* reconstitution [80,81] but also *in vivo*, depending on the availability of Chl *b* [82]. The two luteins are accommodated in two binding sites located in the center of the molecule called L1 and L2, while neoxanthin and violaxanthin are located at the periphery of the complex in sites N1 (near the C helix) and V1, respectively (see Fig. 3B) [83,84]. The binding sites are quite selective for the different xanthophylls [85,86], but the molecular basis for this selectivity has not been fully elucidated yet. The structure also reveals that the average distance between the Chls is around 10 Å. This leads to excitonic interactions between the pigments that result in fast energy transfer in the complex.

Sequence analysis shows high similarity between the members of the Lhc family, thus suggesting a similar structural arrangement for the minor antenna complexes [87]. Moreover, all the amino acids that are Chl-binding residues in LHCII are conserved in all members of the family, with only a few exceptions [88]. Despite the structural similarity, the individual antenna complexes show different biochemical and spectroscopic properties. Part of these differences are due to the fact that the pigment composition is not identical [89] both regarding the Chl *a*/*b* ratio and the carotenoid composition. The pigment binding of most of the complexes was studied with the use of mutation analysis. Mutations of the putative Chl-binding residues followed by *in vitro* reconstitution [90] has led to the production of complexes lacking individual chromophores, allowing the characterization of each chromophore in each binding site [91–96]. This analysis has revealed that the biochemical and spectroscopic properties of Chls in several of the binding sites are conserved across the Lhc family. For example, the four Chl-binding sites located in the center of the molecule (602, 603, 610 and 612, nomenclature according to [78]) accommodate Chl *a* in all antenna complexes, with Chls 602–603 absorbing around 675 nm and Chl 610–612, absorbing around 680 nm, thereby representing the lowest energy state of the system [92,94]. On the other hand, the domain including helix C has a higher tendency to coordinate Chl *b* [92,97], although the occupancy of some of the sites by either Chl *a* or Chl *b* varies for the different complexes and it has been suggested that this difference is mainly related to be possibility/impossibility for the formyl group of Chl *b* to form H bonds [96], thus stabilizing the Chl *b* binding. While the L1 site of all complexes coordinates lutein, the L2 site accommodates lutein in LHCII and CP26, and violaxanthin in CP29 and CP24. Neoxanthin is present in the N1 site of all complexes but CP24 [98].

4.2. Energy transfer in the antenna complexes of Photosystem II

EET in LHCII has been studied extensively in the nineties [99–103]: energy transfer from the carotenoids to the Chls and from Chl *b* to Chl *a* appear to be highly efficient and mainly occur on a subps time scale whereas some transfer processes occur on a time scale of several ps, especially between Chl *a* molecules both within the same and between neighboring monomeric subunits. By combining these results with those of polarized steady-state spectroscopy (e.g. [104]) and mutational analysis [92] it could be concluded that the ps spectral equilibration leads to a situation in which the excitations are very rapidly to a large extent located on the Chl *a* pigments on the stromal part of the protein and preferably at the periphery [105], namely on Chls 610–612, from where they can be transferred to other complexes in the thylakoid membrane. Spatial equilibration within the trimers might occur on a slower time scale (tens of ps) as was concluded from a singlet–singlet annihiliation study [106] and a pressure-induced quenching study [107], whereas (polarized) pump–probe measurements also revealed such "slow" kinetics [100,108,109].

The results of the various time-resolved and steady-state spectroscopic studies were modeled with the use of Redfield theory [110,111] and in 2005 this led to a theoretical description of the data that was to a large extent consistent with the crystal structure [78,79]. The resulting overall picture of the energy flow within LHCII was the same as the previous one [105], meaning that within a few ps the excitations arrive on the lowest-energy states, associated with Chls 610, 611 and 612 on the stromal side at the periphery of the trimer.

More recently, Calhoun et al. [112] have studied trimeric LHCII with the use of two-dimensional electronic spectroscopy and 14 electronic energy levels were determined and they were assigned to excitonic states in a monomeric subunit of LHCII. The results are qualitatively in agreement with those of Novoderezhkin et al. [111,113] although there are several differences in exciton positions and peak amplitudes. Therefore, a new model was developed and the site energies were adjusted to give better agreement with the 2-D data. However, it was not reported whether these modifications also lead to a correct description of for instance the linear dichroism spectrum [104], which was crucial for the modelling of Novoderezhkin et al. [111] and the circular dichroism spectrum [114]. In another recent study by Schlau-Cohen et al. [115] a

sub-110 fs relaxation process through spatially overlapping states was detected that had not been observed before but all the slower processes that were observed were in excellent agreement with previous results. It should be noticed that although all theoretical models are based on the structural model of Lhcb1, the (sub)ps transient absorption kinetics of three gene products forming LHCII, Lhcb1, Lhcb2 and Lhcb3, are identical [116].

EET in the minor antenna complexes [117–123] seems to follow similar internal pathways as in LHCII. Although differences have been observed on a fast time scale, also in these complexes the equilibration occurs within a few ps, leading to the population of the lowest energy forms, which in all complexes are located in the stromal domain, mainly on Chls 611 and 612 [124].

5. PSII supercomplexes

5.1. The variable structure of PSII supercomplexes

The association of the antenna complexes with the PSII core in plants is relatively labile, making it difficult to obtain homogeneous preparations of PSII supercomplexes. Even upon very mild detergent treatment, i.e. in conditions in which PSI-LHCI remains fully intact, PSII supercomplexes disassemble quite easily [84,125]. The largest supercomplex purified so far is called C2S2M2 (Fig. 1A) [126] and it is the most abundant complex present in the membranes of *Arabidopsis thaliana* [127]. It is composed of a dimeric core (C2), 4 LHCII trimers and 2 copies of the monomeric complexes CP29, CP26 and CP24 [128]. Cross-linking experiments and analysis of mutants lacking individual subunits have shown that CP29 is located near CP47, while CP26 is near CP43 [129,130]. The two LHCII trimers that are present per monomeric core differ in subunit composition and interaction strength (S = strongly bound; M = moderately bound) with the rest of the supercomplex. The S trimer is located on the CP26 side; it is composed of the products of the Lhcb1 and Lhcb2 genes [131] and it is strongly connected with the core. The M trimer is attached to the supercomplex on the side of CP29 and CP24 [132] and in addition to Lhcb1 and Lhcb2 it also contains the product of the Lhcb3 gene [128]. Smaller supercomplexes (C2S2) lacking trimer M entirely, have been observed upon solubilization of the membranes of different species [133,134]. They can arise from C2S2M2 after the loss of the M trimers, but ordered arrays of C2S2 have also been observed in the membranes of spinach [134] and pea [135]. On the other hand, ordered arrays of C2S2M and C2S2M2 supercomplexes have been observed in *Arabidopsis* [130] and in spinach [134], respectively (see below).

In addition to C2S2 and C2S2M2, PSII supercomplexes with different antenna composition, like C2S2M, C2SM, C2M2 and CS, have been purified [126]. Probably these complexes arise from a partial disassembly of the larger system, although it can not be excluded that some of them also exist in the membrane.

Recently a projection map at 12 Å resolution has been obtained for the C2S2M2 supercomplex, which has allowed to determine the orientation of the individual subunits in the supercomplex [126]. Based on these results the 3D structure of the supercomplex (Fig. 1A) has been reconstructed using the crystal structures of the core and of LHCII. This model allows to visualize the organization of the pigments in the supercomplex (Fig. 1B) and thus to propose possible EET pathways (see below).

5.2. Role of the individual antenna complexes in the structural organization of PSII

In the antisense mutants of CP29, no PSII–LHCII supercomplexes could be found upon mild detergent solubilization, indicating that CP29 plays a crucial role in the assembly and stability of the supercomplexes [136]. The recent finding that a stable sub-complex composed of a monomeric core, CP26 and LHCII-S could be isolated, suggests a role for CP29 in the stabilization of the dimeric complex [126]. Indeed in dimeric PSII, CP29 is in contact with the trimer S that is associated with a different monomeric core complex. On the contrary, the absence of CP26 does not seem to have any effect on the assembly, in agreement with the fact that PSII supercomplexes lacking CP26 were also found in wild-type plants [130,137]. CP24 is present only in the $C_2S_2M_2$ complexes, which are actually only formed when CP24 is present [138], thus confirming the interactions between this subunit and the M trimer. The lower amount of NPQ of CP24KO plants and the fact that in light stress CP24 dissociates from CP29 and LHCII, also suggests a role for this subunit in photoprotection [138–140].

Of the three Lhcb genes encoding for LHCII subunits, Lhcb3 is only present in higher plants which is also the case for CP24 [141] and a direct correlation has been observed between the presence of these two complexes, suggesting that they are involved in the docking of trimer M to the supercomplex [126]. Although EM analysis of the membrane shows that C2S2M2 supercomplexes are formed in the absence of Lhcb3 [142], these supercomplexes do not survive purification [126] in contrast to the situation in WT plants.

The fact that upon mild detergent solubilisation CP24 and CP29 are mainly present in a complex that is composed of LHCII–CP24–CP29 (giving rise to a band in a sucrose gradient that is called band 4)[132] indicates that the interactions between these three subunits are stronger than the interactions of CP24 and CP29 with the core. The analysis of an antisense mutant of Lhcb2, in which also the level of Lhcb1 is strongly reduced, shows that CP26 is able to substitute for these two subunits and is able to form trimers together with Lhcb3, leading to PSII supercomplexes which are indistinguishable from the WT complexes [143]. Remarkably, mild solubilisation from the Lhcb2as mutant in which CP26 replaces trimeric LHCII, still leads to a large amount of "band 4" [140].

5.3. Excitation energy transfer and charge separation in PSII supercomplexes

Recently, picosecond fluorescence measurements have been performed on four different Photosystem II (PSII) supercomplexes purified from *Arabidopsis thaliana* [144]. The main difference between these supercomplexes concerns the size of the outer light-harvesting antenna [126]. The smallest complex (C2S) contains a dimeric PSII core plus one LHCII trimer, CP26 and CP29. The largest complex contains four LHCII trimers and two copies of CP24, CP26 and CP29 per dimeric core (C2S2M2). The average fluorescence lifetime increases upon increasing the antenna size from 123 ps for C2S to 155 ps for C2S2M2 in the presence of 0.01% of the detergent α-DM. In the presence of 0.001% α-DM, these lifetimes were somewhat shorter, ranging from 104 ps to 143 ps. The analysis of these data is currently in progress (Caffarri, Broess, Croce and van Amerongen, in preparation) but it appears that the connectivity between the complexes becomes better when the size of the antenna increases. In this respect it is interesting to note that the average lifetime of the core preparation from maize [76] was somewhat larger than 100 ps, which is similar to the value for C2S in [144] that contains significantly more pigments, in line with the hypothesis that additional complexes might help to improve the connectivity, although one might also argue that the charge-separation process speeds up in the presence of extra antenna complexes. This was in fact proposed by Engelman et al. [77] when the average lifetime of core preparations and intact thylakoid membranes were compared. However, in that case the average lifetime of the core was extremely long (around 170 ps) and the integ-

rity of the core complexes might thus be questioned. The average fluorescence lifetime of the largest supercomplex in 0.001% α-DM is slightly shorter than the average lifetime of 150–160 ps that was obtained for PSII membranes [42,145], containing somewhat more LHCII (2.45 instead of 2.0 trimers per RC). This suggests on the one hand that the isolated supercomplexes maintain the properties that they have in the membrane and on the other hand that the supercomplex is the functional unit of the membranes.

6. Energy transfer and charge separation in PSII membranes

6.1. PSII organization in the grana membranes

The thylakoid membrane can morphologically be divided into two parts: the grana, which are composed of stacks of membrane disks, and the stroma lamellae, which connect the grana [135,146–149]. Photosystem I and Photosystem II are laterally segregated with the former being present in the stroma lamellae (together with the ATP synthase) and the latter mainly in the grana [150].

Grana membranes and subfractions of grana membranes can be purified and they were shown to contain practically only PSII complexes [151–153]. However, it is not completely understood how the complexes are organized in the membrane. The issue is complicated by the fact that the membrane is flexible and dynamic and able to respond to changes in environmental and growth conditions apparently by changing not only the composition but also the organization of the PSII supercomplexes [154–156]. It has been shown that part of the grana membrane is occupied by PSII arrays composed of supercomplexes with different antenna sizes (see above), but the abundance of the arrays seems to depend on the species analysed and on the growth conditions [134,135,149,157]. It is generally accepted that only part of the PSII supercomplexes is embedded in these regular arrays, while another part is present in the membrane in a less organized fashion. It is not clear what the role of the arrays is. It has been suggested that they facilitate the diffusion of small molecules [157] like plastoquinone, but it has also been suggested that they have the opposite effect of slowing down the diffusion [139] in the membrane.

It has also been proposed that C2S2 represents the supercomplex in high light, while C2S2M2 is the result of low-light growth [135]. Although in high light the amount of LHCII trimers is indeed lower than in low light, in all cases the stoichiometry LHCII/core is higher than 2 (it is usually between 3 and 4) [42], meaning that C2S2M2 supercomplexes in principle could still represent the minimal PSII unit in all conditions but also indicating the presence of extra trimers somewhere in the thylakoid membrane. Where are these trimers located? It has been proposed that part of the LHCII antenna is located in different membrane layers containing only LHCII trimers [158]. However, recent tomography results seem to exclude this possibility [135,149]. It has also been proposed that LHCII can exist in the membrane in the form of oligomers and such oligomers (heptamers) have been observed by electron microscopy [159] although this finding has recently been challenged [160]. The location of the "extra" LHCII timers thus remains an open question. If LHCII-only regions indeed exist, these complexes should still be able to transfer excitation to the RC's or these complexes should be highly quenched, otherwise long fluorescence lifetimes (many ns) should be observed in time-resolved fluorescence measurements on PSII membranes or thylakoid membranes, in contrast to experimental observations.

6.2. Energy transfer in the grana membranes

In the past various studies have been performed on PSII membranes (so-called BBY preparations [151]). The kinetics in these membranes were for instance described by a single lifetime of 210 ps [161] or with a major lifetime of 140 ps and a minor lifetime of 330 ps [162]. Recently, two studies were performed that showed average lifetimes in the order of 150–160 ps [42,145] and the results were interpreted with a coarse-grained model that uses the C2S2M2 structure as a basis. In this model it was assumed that primary charge separation (with rate k_{CS} or inverse rate/transfer time τ_{CS}) is reversible (first charge-separated state is ΔG lower in energy than the state in which the RC is excited in the Q_y state). Secondary charge separation (with rate k_{RP} or inverse rate/transfer time τ_{RP}) was supposed to be irreversible. EET was modelled by assuming hopping to occur between neighboring (monomeric) complexes with a rate called k_H (or inverse rate/hopping time τ_H) that was assumed to be the same for all hopping steps, whereas each rate was scaled with the number of pigments per complex. The basic difference with the earlier ERPE model is the fact that the supercomplex is used as a structural model to include EET steps and the fact that the hopping rate is not assumed to be infinitely fast. Using this model it was shown that different combinations of τ_{CS} and τ_H can describe the data nearly equally well [42]. Although it was not possible to extract more details about the charge-transfer kinetics in the RC, it was nevertheless possible to verify/falsify whether existing models for the charge-separation kinetics in the RC were in agreement with the data on the BBY particles. For instance, it turned out to be impossible to model the data on the BBY samples with the models for charge separation as obtained from time-resolved studies on cores by Vasilliev et al. [65] and Miloslavina et al. [67], irrespective of the chosen rate for k_H. Good resemblance could only be obtained when both the rate of charge separation and the drop in free energy upon charge separation were increased. Although these features are reminiscent of the outcome of the theoretical work on the PSII core of Raszewski and Renger [66], it remains to be tested how good the agreement is. It was also tested to which extent charge-separation models based on measurements on isolated PSII RC [6,71] were in agreement with the BBY results. These models cannot be used directly because the isolated RC's do not contain the electron acceptor Q_A. But whatever value was assumed for the rate of electron transfer to Q_A, no agreement with the BBY data could be obtained as all the simulations based on the models for isolated RC's led to decay kinetics that were too slow. Again, it was only possible to improve the resemblance with the experimental data by speeding up the charge-separation process and increasing the drop in free energy. Although only the models presented in the papers of Andrizhiyevskaya et al. [71] and Groot et al. [6] were explicitly tested, other results that were presented in literature [67,70–73] will give similar (non-successful) results because none of them show the required fast primary charge separation accompanied by a large drop in free energy. At the moment it is unclear what the reason is for this discrepancy, although it is has been proposed in the past (but also disputed) that isolation of the RC's leads to modifications of their performance that may cause an increase of the effective charge separation time.

As was mentioned above, different combinations of τ_{CS} and τ_H can describe the BBY data equally well, using the coarse-grained modelling approach [42]. When for the hopping rate a value was taken that was in agreement with results on random aggregates of LHCII [106], it had to be concluded that τ_{mig} was around 100 ps and was dominating the overall trapping process. However, in [145] time-resolved fluorescence measurement were performed on PSII membranes, using two different excitation wavelengths in order to vary the relative number of excitations in the core and the outer antenna. At 483 nm 86% of the excitations were created in the outer antenna and at 420 nm this number was 68%, and the latter case corresponded to a slightly faster average fluorescence decay time (by 4.2 ± 1.8 ps), implying that the migration time from

outer antenna to core is around 20–25 ps. Using the same coarse-grained model as in [42] the following values were estimated: τ_{hop} = 3.5 ± 0.9 ps, τ_{CS} = 5.5 ± 0.4 ps, τ_{RP} = 137 ± 5 ps, ΔG = 826 ± 30 cm^{-1}, which implies trap-limited kinetics. However, it should be realized that in the modelling the same hopping rate was assumed for transfer from CP47/CP43 to the RC as for transfer between all the other complexes which leads to a migration time to the RC of 35 ps. As was discussed extensively above, the rate of transfer from CP47/CP43 to the RC is still under discussion and therefore the consequences and the correctness of this assumption require further study in the future.

As was also mentioned above, the migration time from the outer antenna to the core would take around 100 ps instead of 25 ps if EET in the outer antenna would occur with the same speed as EET in random aggregates of LHCII [42,106]. This indicates that some sort of optimization has been achieved in organizing the light-harvesting complexes in the supercomplexes/PSII membranes in such a way that efficient EET takes place. Therefore, it is of interest to have a closer look at the organization of the supercomplexes.

Based on the projection map of C2S2M2 from *A. thaliana* at 12 Å resolution a model of the 3D structure of the supercomplex (Fig. 1) was reconstructed [126] using the crystal structures of PSII core [44] and LHCII [78]. For the minor antenna complexes, the structure of a monomer of LHCII was used while the pigment composition/occupancy was assigned based on the results of mutation analysis experiments on *in vitro* reconstituted complexes [91,92,95,96]. The model shows that LHCII S is connected directly with CP43 and that Chls 612/611 of one of the monomers are facing Chl 43 of CP43. The excitonic pair 612/611 harbors the lowest energy site of all Lhcb complexes [92,124], thus being the most populated form at Boltzmann equilibrium, thereby facilitating a fast flow of excitation energy from the periphery to the core. CP26 is also organized in a similar way, with Chls 612/611 facing Chl 49 of CP43. In between CP26 and LHCII there is mainly Chl 605 which accommodates a Chl *b* molecule [78], while all other Chls seem to be at a relatively large distance. This suggests that EET between LHCII and CP26 is very slow. The model also shows that there is no direct contact between LHCII-M and the core, and EET thus has to occur via CP29. LHCII-M is also connected with CP24 but according to the model, CP24 does not deliver excitations to the core directly but only via CP29. It should however be considered that in the model the core from cyanobacteria has been used, which differs from the core of higher plants regarding the presence/absence of a few small subunits. It can not be excluded that in the plant core an extra subunit coordinating Chls is present in between CP24 and CP47, thus allowing direct EET from CP24 to core. The presence of a direct connection between CP24 and core would also speed up the transfer from LHCII to the core. Looking at the Chl distribution in the supercomplex (Fig. 1B), there seem to be EET pathways from the periphery (LHCII) to the RC via closely spaced Chl *a* molecules, bypassing the Chls *b* that belong to different complexes and that are clustered together. The presence of Chl *a* EET pathways must be responsible for the efficient energy transfer in PSII supercomplexes as compared to random aggregates of LHCII.

7. Energy transfer and charge separation in PSII in the thylakoid membrane

The thylakoid membrane can be considered as the minimal unit in which all complexes participating in the light reaction of photosynthesis are still present, thereby representing a good system for mimicking the *in vivo* situation. Moreover, recent results strongly suggest that acclimation mechanisms involve reorganization at the level of the membranes (e.g. [140,155,163]) and thus for a full understanding, acclimation studies should be performed at the membrane level. However, the large heterogeneity of the system and the presence of different complexes strongly complicate the analysis of the data. To disentangle the contributions of different complexes and to relate these to the composition and organization of the membrane thus represents a very important task to allow future studies on stress and acclimation responses, also *in vivo*.

In general, the kinetics of thylakoid membranes are multi-exponential with lifetimes ranging from tens of picoseconds to values between 300 and 600 ps, whereas the average lifetime generally ranges from 300 to 400 ps [77,164–166]. However, agreement was never reached about the assignment and interpretation of all the obtained lifetime components, (for an overview see also [36,167]). An additional complication in studies on photosynthetic membranes and chloroplasts is the fact that the thylakoid membranes are heterogeneous and contain both PSI and PSII with their spectra heavily overlapping and reaction kinetics partly occurring on similar time scales [168], making it difficult to distinguish between the various processes taking place [169]. Recently, thylakoid membranes from *Arabidopsis thaliana* with 4 LHCII trimers per RC were studied with time-resolved fluorescence, using various detection wavelengths to discriminate between the kinetics of PSI and PSII and different excitation wavelengths to estimate the contribution of the migration time of excitations of the outer antenna of PSII to the core [170]. The fluorescence decay could be fitted very well with three lifetimes, 73 ps, 251 ps, 531 ps (plus a very small contribution of a ns component) at all wavelength combinations but with varying amplitudes. The average fluorescence lifetime of PSII was found to be 326 ± 2 ps upon excitation at 412 nm and 339 ± 1 ps after 484 nm excitation. Using the charge separation parameters given above [145] it was calculated that τ_{trap} = 180 ps and τ_{mig} = 150 ps (note that here τ_{del} is included in τ_{mig}). This migration time is a factor of 4–5 larger than for the PSII membranes which contained 2.4–2.5 trimers per RC. Therefore, it is clear that the extra trimers are connected less well to the RC's. This is in disagreement with the conclusion of Barter et al. [171] that the average excited-state lifetime scales with the number of pigments, which was taken as proof for trap-limited excited-state kinetics. The results of van Oort et al. [170] indicate that at the level of the thylakoid membrane trap-limited models are not valid anymore.

The same experiments were also performed on thylakoids from mutants that were lacking either CP24, CP26 or CP29 but contained identical amounts of LHCII, i.e. four trimers per RC. The absence of CP26 led to a small decrease of the average lifetime for both excitation wavelengths, demonstrating that the connectivity between the various complexes is still equally good as in WT, in agreement with the EM and biochemical studies [172]. In the absence of CP29 the connectivity in PSII appeared to be disturbed to some extent, although it is still rather good. This might seem to be at variance with the results of EM and biochemistry [172], that show that the supercomplexes are strongly destabilized in the absence of CP29. Apparently, this does not prohibit relatively good connectivity between the subunits. In the absence of CP24 the difference in lifetimes for the two excitation wavelengths was quite large: 329 ps (412 nm) 413 ps (484 nm) and it was concluded that the connection between part of the antenna and the RC's was substantially disturbed. Indeed with EM measurements [138] two different regions could by identified in the membrane: (i) microcrystalline arrays composed of highly ordered C2S2 supercomplexes; and (ii) regions strongly enriched in LHCII. Thus, in CP24ko fast energy transfer is expected within the C2S2 supercomplexes, whereas a slower lifetime should be associated to the regions enriched in LHCII, thus explaining the large difference in lifetime observed after excitation at different wavelengths. The fact that the lifetimes are still similar to those of WT *Arabidopsis* despite the bad connec-

tions was explained by strong excited-state quenching of the badly connected light-harvesting complexes.

8. Conclusions and future outlook

It is clear from the above discussion that a lot of questions about the organization and functioning of PSII remain. How is PSII organized in the membranes? How does this depend on the species or on the growth conditions? How does the organization affect the functioning? What is the role of the arrays? And do the arrays contain active or inactive PSII? At the moment it is not completely clear to which extent the structure of PSII core from cyanobacteria and plants are identical but it seems that core preparations from plants show slower excited-state decay kinetics. The interpretation of core kinetics in general differs considerably in different studies. Some experimental studies seem to indicate that excitation energy transfer from CP43 and CP47 to the RC is much faster than the overall trapping time (trap-limited kinetics) but this seems to be in disagreement with theoretical calculations. If on the other hand "reasonable" slow transfer rates are assumed (transfer-to-the-trap limited), the rate of charge separation should become much faster than has been observed for isolated RC's and the accompanying drop in free energy should be much larger than has been observed. However, time-resolved fluorescence results on PSII membranes seem to indicate that charge separation is faster in the membrane than in isolated cores and RC's.

More agreement exists about the excited-state dynamics of the outer antenna complexes. In trimeric LHCII excitations flow on a time scale of a few ps mainly to the Chl *a* molecules at the periphery on the stromal side and the same is happening in the strongly homologous minor antenna complexes CP24, CP26, CP29. Transfer between different complexes also occurs on a fast time scale and in PSII membranes EET from the outer antenna to the core occurs in around 20–25 ps, and the rate of transfer is a factor of ~5 faster than in random aggregates of LHCII. How the EET can be so fast in the supercomplexes is not understood yet but it is clear from the recent structural model that specific pathways exist (consisting of connected Chl *a* molecules). In thylakoid membranes where the average number of LHCII trimers goes up from ~2.5 to 4, the migration time is considerably slower, demonstrating that on the thylakoid level the charge-separation process is definitely not trap-limited. It is still not known where the extra antenna complexes are located but it is also not known to which extent they are disconnected and to which extent the complexes are quenched.

It is clear that a consistent description of the kinetics ranging from PSII RC's to PSII in the thylakoid membrane has not been obtained yet, and such a description is not only needed to understand the CS and EET processes in PSII but it will also be needed to interpret picosecond (micro)spectroscopic measurements on photosynthetic organisms *in vivo* that will be needed to study acclimation and stress processes in detail.

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